Towards a High-Throughput New Approach Method for Acute Fish Toxicity: Painting the Rainbow (Trout)

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State of the art

In vitro methods in ecotoxicology

- *In vivo* chemical hazard evaluations are cost and time intensive and low throughput.
- High-throughput *in vitro* methods for ecotoxicology are needed to accelerate the pace of chemical hazard evaluation.
- A lower throughput *in vitro* method using a rainbow trout gill cell line (RTgill-W1) has been developed by Schirmer et al. 1998 and Tanneberger et al. 2013.
  - adopted by the OECD (OECD TG 249).
  - Upon adjustment of assay results for *in vitro* disposition and conducting in vitro-to in vivo-extrapolation (IVIVE), excellent correlation with *in vivo* survival data was found (Tanneberger et al. 2013)

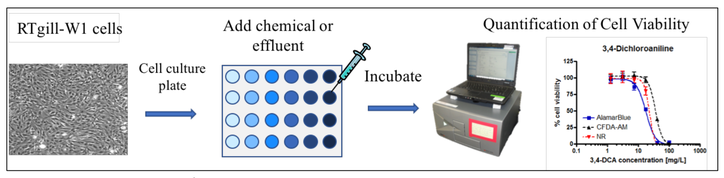

Illustration: www.aquatox-solutions.ch

Want to hear more about RTgill-W1? visit
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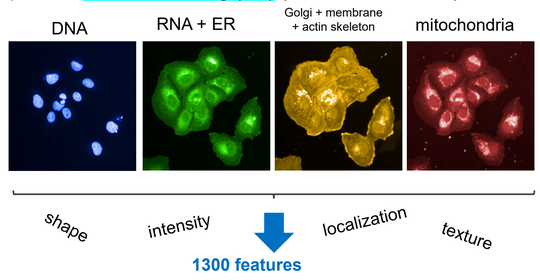
High-throughput method with rainbow trout gill cells

High-throughput methods in human toxicology

- New Approach Method (NAM): any approach that does not use intact animals
- The US EPA has implemented two high-throughput profiling methods: transcriptomics and Cell Painting (CP) (Thomas et al. 2019)



- CP is cost-effective and is amenable to any type of adherend cells
- We have screened >1000 chemicals in human osteosarcoma (U-2 OS) cells (Nyffeler et al. 2020, Nyffeler et al., *in press*)

Aims & Conclusions

1. Miniaturize the existing OECD TG249 (CV-PR) assay to 384-well format.

⇨ **With minor modifications, OECD TG249 could be miniaturized.**

2. Apply imaging-based Cell Viability (CV-IB) and Cell Painting assays in RTgill-W1 cells.

⇨ **RTgill-W1 cells were amenable to the CV-IB and CP assays.**

3. Test 227 chemicals of interest in all three assays and compare the resulting potency estimates among the three assays.

⇨ **52% of chemicals were active in at least one assay. The CP assay was more sensitive than the CV assays.**

4. Develop a high-throughput workflow to extrapolate the *in vitro* data and compare it to *in vivo* literature data.

⇨ **For 57% of chemicals the predicted value was within 10x of *the in vivo* mortality data.**

Overview

High-throughput bioactivity screening

- 384-well format
- 227 unique chemicals
- 8 concentrations per chemical
- 4 biological replicates (independent cultures)
- 3 assays:
  - Plate reader based-cell viability (CV-PR)
    - Alamar blue
    - CFDA-AM
    - Neutral Red
  - Image-based cell viability (CV-IB)
    - number of cells
    - % propidium iodide positive cells
  - Cell Painting (CP)
    - global Mahalanobis distance

nominal *in vitro* potency

published *in vitro* data

***In vitro* disposition**

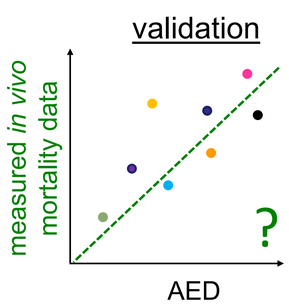
validation

analytical chemistry

adjusted *in vitro* potency

Reverse toxicokinetics

administered equivalent dose (AED)



High-throughput bioactivity screening

Summary of screening results

227 unique chemicals were tested in all 3 assays:

A

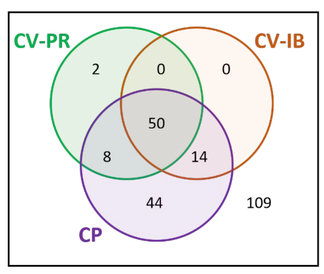


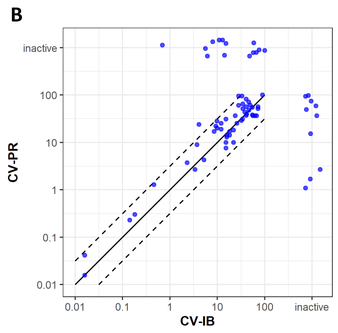
***Fig. 1. A:** Venn diagram of chemicals active in each assay. **B:** Scatter plot of the nominal benchmark concentration [in uM] of chemicals active in either assay.*

➔**52% of chemicals were active in at least one assay**

➔ **22% of chemicals were active in all three assays**

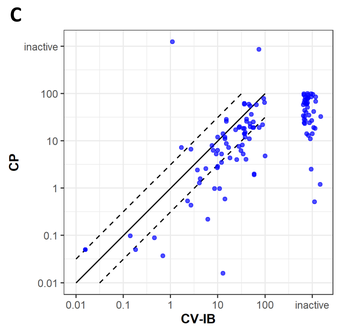
➔**51% of chemicals were active in HTPP**

B



➔**Good correlation of in vitro potencies between the two CV assays**

C



➔**CP potencies are slightly more sensitive than CV potencies**

➔**Many chemicals active in CP that were inactive in CV**

Example: 6PPD and 6PPD-quinone

- Out of all tested chemicals, 6PPD-quinone has one of the lowest BMC values.
- 6PPD is an antiozonant added to rubber in tires, which is oxidized to 6PPD-quinone
- 6PPD-quinone in motorway runoff has been implicated as a causative agent for coho salmon die-offs in the Puget Sound (Tian et al 2022).

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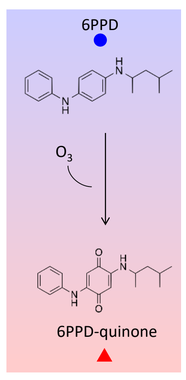

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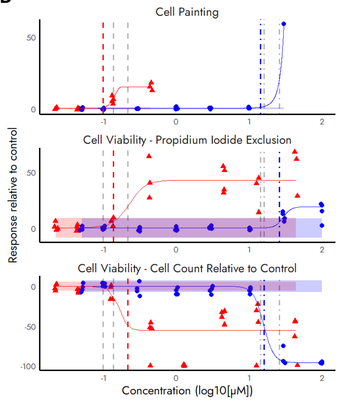

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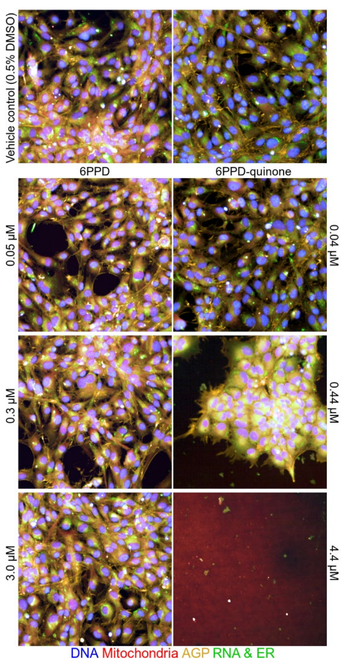


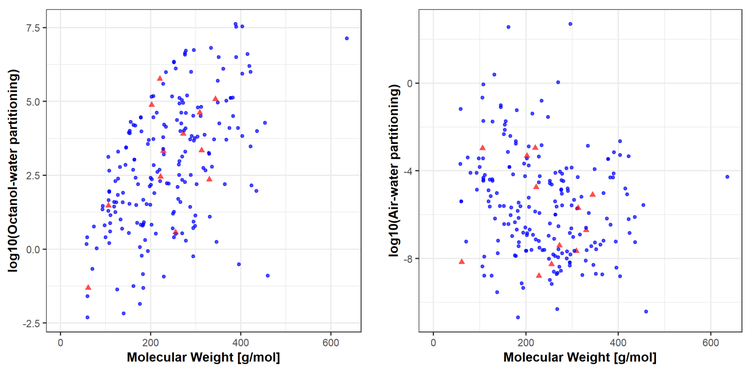
***Fig. 2. A:** Oxidation of 6PPD to 6PPD-quinone. **B:** Concentration/response curves for Cell Painting and CV-IB data. Vertical lines represent benchmark concentration (BMC) values for each endpoint: All three endpoints are shown in each facet for comparison, but the BMC value corresponding to each endpoint is highlighted in red or blue on the respective graphs. **C:** Representative composite images at comparable nominal concentrations of 6PPD (left) and 6PPD-Q (right), compared to vehicle control (top).*

➔ **For RTgill-W1 cells, 6PPD-quinone was approximately 100x more potent than 6PPD in both Cell Painting and CV assays.**

In vitro disposition modeling

Diversity of the chemical set

Predicted physico-chemical properties of 209 tested chemicals. The 12 chemicals selected for experimental validation are highlighted in red.



➔**The 12 chemicals for experimental validation cover a large range of physico-chemical properties**

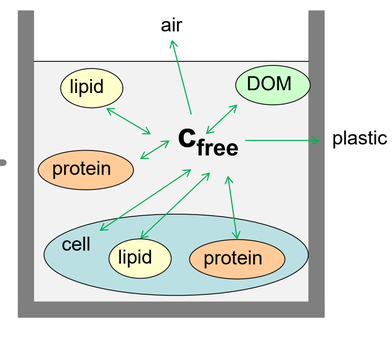
In vitro disposition modeling

Armitage 2021 model

geometric parameters
- well volume
- well surface

test-system specific parameters
- protein & lipid content of cells
- protein, lipid and DOM content of medium

chemical-specific parameters
- phys-chem properties



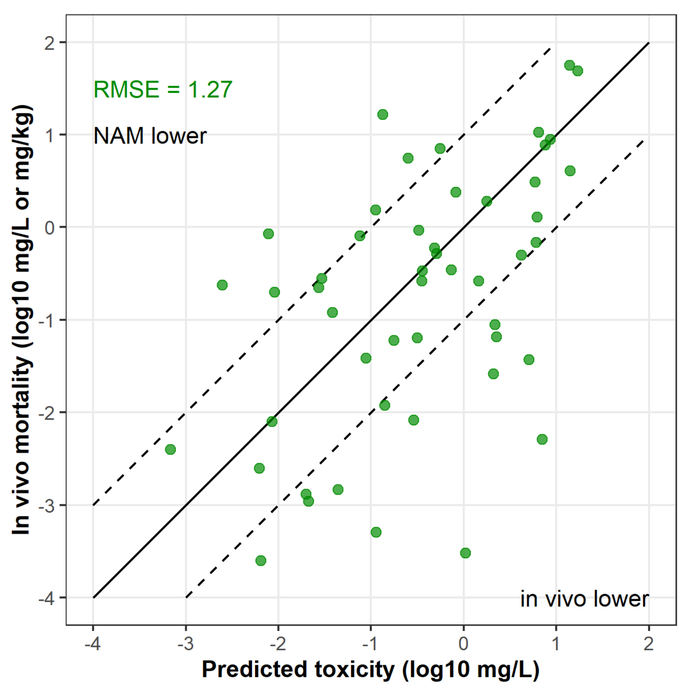
Comparison to *in vivo* mortality data

ECOTOXdb Knowledgebase
https://cfpub.epa.gov/ecotox/

- Species:
  - Rainbow trout
  - Fathead minnow
- Endpoint: Mortality

92 chemicals with ≥ 3 records

49/92 chemicals were active in the screen



➔**For 28/49 (57%) of chemicals, toxicity was predicted within 10-fold of the *in vivo* data.**

➔**For 36/49 (73%) of chemicals the predicted dose would have been protective (i.e., similar or lower than the *in vivo* value).**

References

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